Latest advances in the vitrification of bovine embryos *in vitro* produced

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1. Introduction

In the last twenty years, the assisted reproductive technologies in the bovine sector suffered changes:

- **Traditionally**: Sperm; *In vivo* derived (IVD) embryos
- **Currently**: *In vitro* produced (IVP) embryos

Cryopreservation: Slow-rate freezing; Vitrification

Benefits of cryopreservation:
- Long term storage
- Genetic selection
- Optimizes reproductive systems
- International exchange
- ↓ Disease transmission

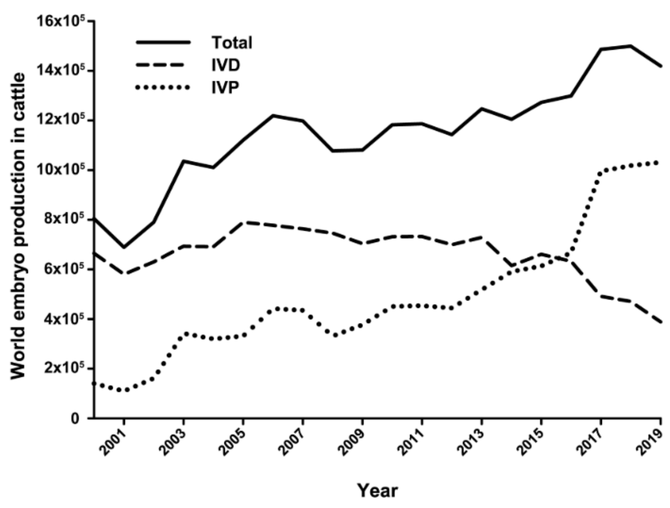


Fig. 1. Number of bovine embryos (*in vivo* derived [IVD], *in vitro* produced [IVP], and total) recorded in the period 2000-2019 (Viana 2020).

While traditional slow-rate freezing has proved successful for the cryopreservation of IVD embryos, vitrification seems to be more efficient for IVP embryos in terms of embryo survival after warming. However, pregnancy rates associated with vitrified IVP embryos are still lower than those obtained with IVD cryopreserved.

Transferred frozen embryos in 2019: 60% IVD; 42% IVP

Objectives

To make an up-to-date compilation through bibliographic research of the **latest advances in the field of vitrification of bovine *in vitro* produced embryos**.

2. *In vitro* production

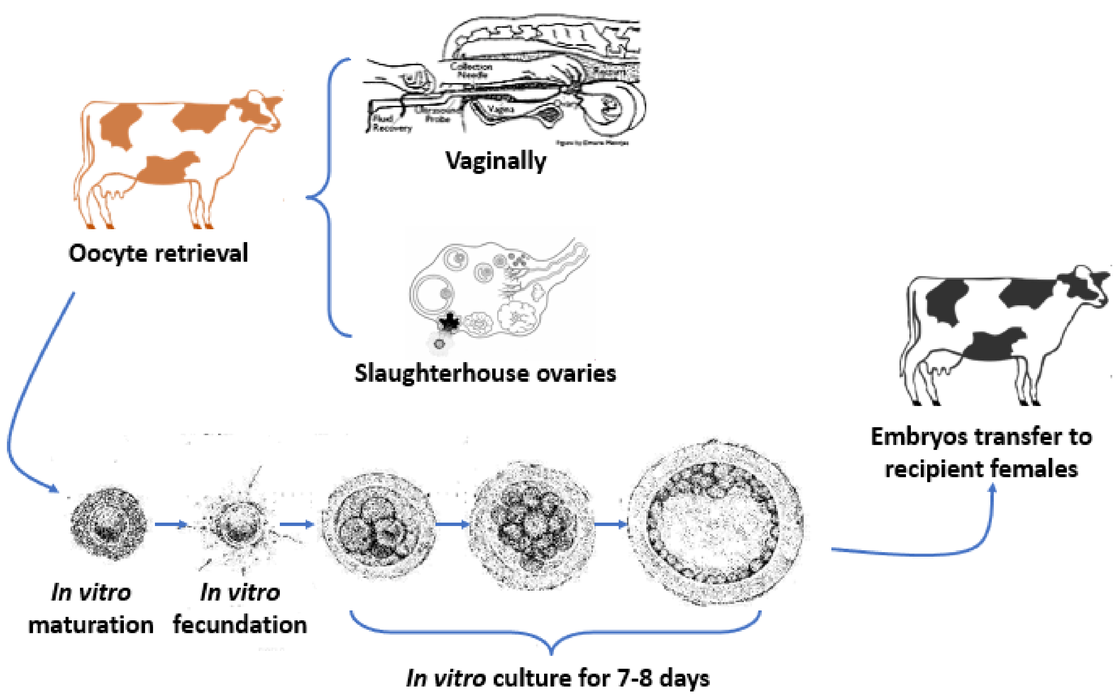


Fig. 2. *in vitro* embryo production system.

3. Vitrification

Slow-rate freezing ↓ [CPA] ✓ Extracellular ice crystal formation

Vitrification ↑ [CPA] ✗ Extracellular and intracellular ice crystal formation

Three factors have to be considered for a successful vitrification and to prevent intracellular crystallization of water:

1. Rapid cooling and warming 2. CPA concentration 3. Media volume

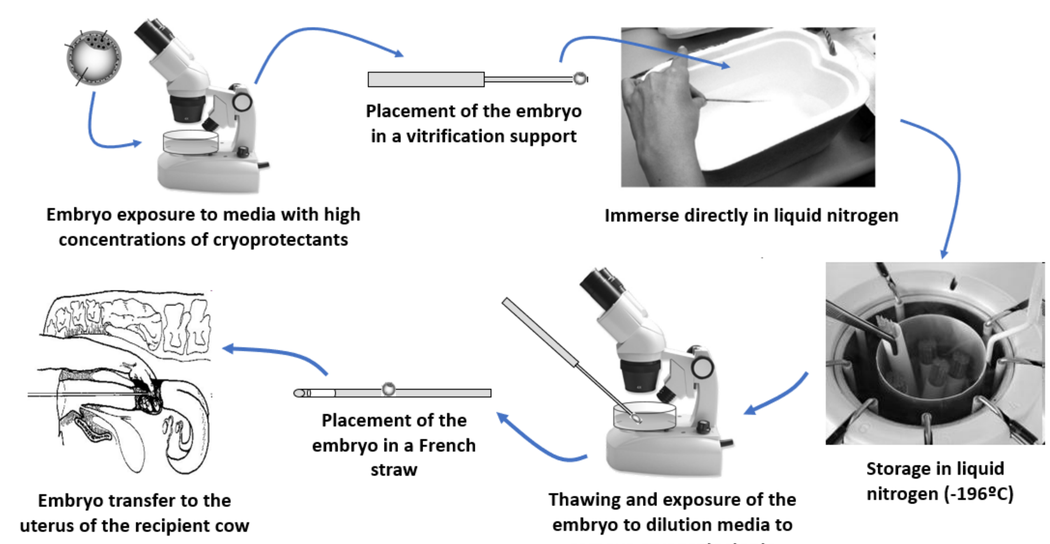


Fig. 3. Embryo vitrification process.

4. New strategies to improve vitrification

5.1. Slush nitrogen

Cooling liquid nitrogen to its freezing point (up to -210 °C) → **SLUSH**

↑ cooling rate 2 to 6 times

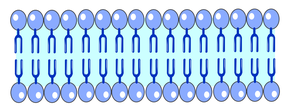
5.2. Cryoprotectants Agents (CPA)

Reduce or prevent freezing damage by:

- Decrease the melting point of water.
- Induce gradual cell dehydration together with the gradual intracellular diffusion of additional permeating CPA through an equilibrium process.

A. Different CPA in the same solution, ↓concentration and toxic effect.
B. Changing the [CPA] and the embryo exposure time.

5.3. Cholesterol

Cholesterol : Phospholipids  Fluidity Stability

The plasma membrane can be enriched with cholesterol by incubating embryos with methyl-β-cyclodextrin loaded with cholesterol.

5.4. Lipolytic agents

- **Forskolin:** adenylate cyclase → lipase
- ***10t,12c*-CLA:** ↓ uptake of fatty acids
- **L-carnitine:**
  - transports fatty acids to mitochondria to generate ATP
  - ↓ROS → protection against apoptosis

5.5. Growth stimulators

- **Leukaemia inhibitor factor:** ↑ development and cryotolerance
- **GF1:** ↑ blastocyst survival

5.6. Antioxidants

- **Resveratrol**
- **Melatonin**

↓ROS → ↓apoptosis

5.7. Anti-freeze proteins

Bind to ❄ → ✗ crystallization

5. Conclusions

- ✓ The simplicity and economic benefits of vitrification could provide an efficient and practical method for the cryopreservation of bovine IVP embryos, significantly improving domestic animal breeding programs.
- ✓ Both embryo survival after warming and gestation rates remain low after the vitrification technique.
- ✓ In order to improve IVP embryo survival after vitrification, further investigation is required; either by modifying IVP protocols or by modifying certain embryo traits to increase their resistance to cryopreservation.